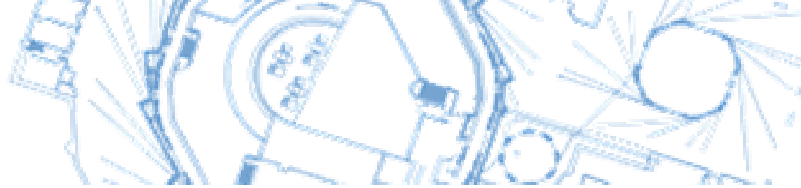
NSLS
NATIONAL SYNCHROTRON LIGHT SOURCE



Infrared Imaging and Microspectroscopy

Lisa M. Miller

National Synchrotron Light Source

Brookhaven National Laboratory

Upton, NY 11973

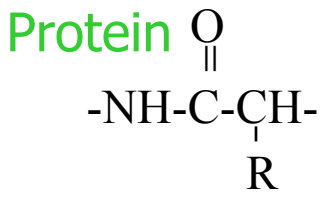
U.S. DEPARTMENT OF ENERGY
OFFICE OF BASIC ENERGY SCIENCES

Funded under contract: DE-AC02-98CH10886

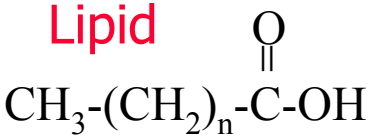
BROOKHAVEN NATIONAL LABORATORY
BROOKHAVEN SCIENCE ASSOCIATES

Chemical Features of Biological Components

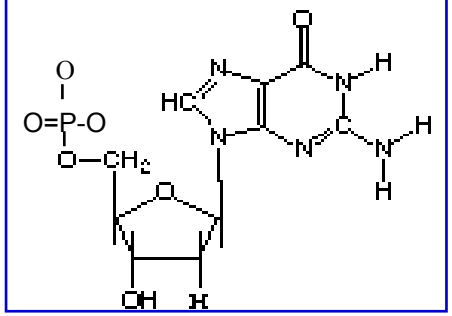
Protein



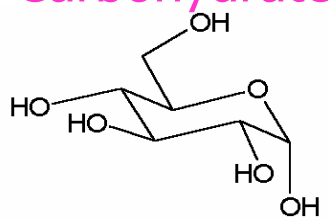
Lipid

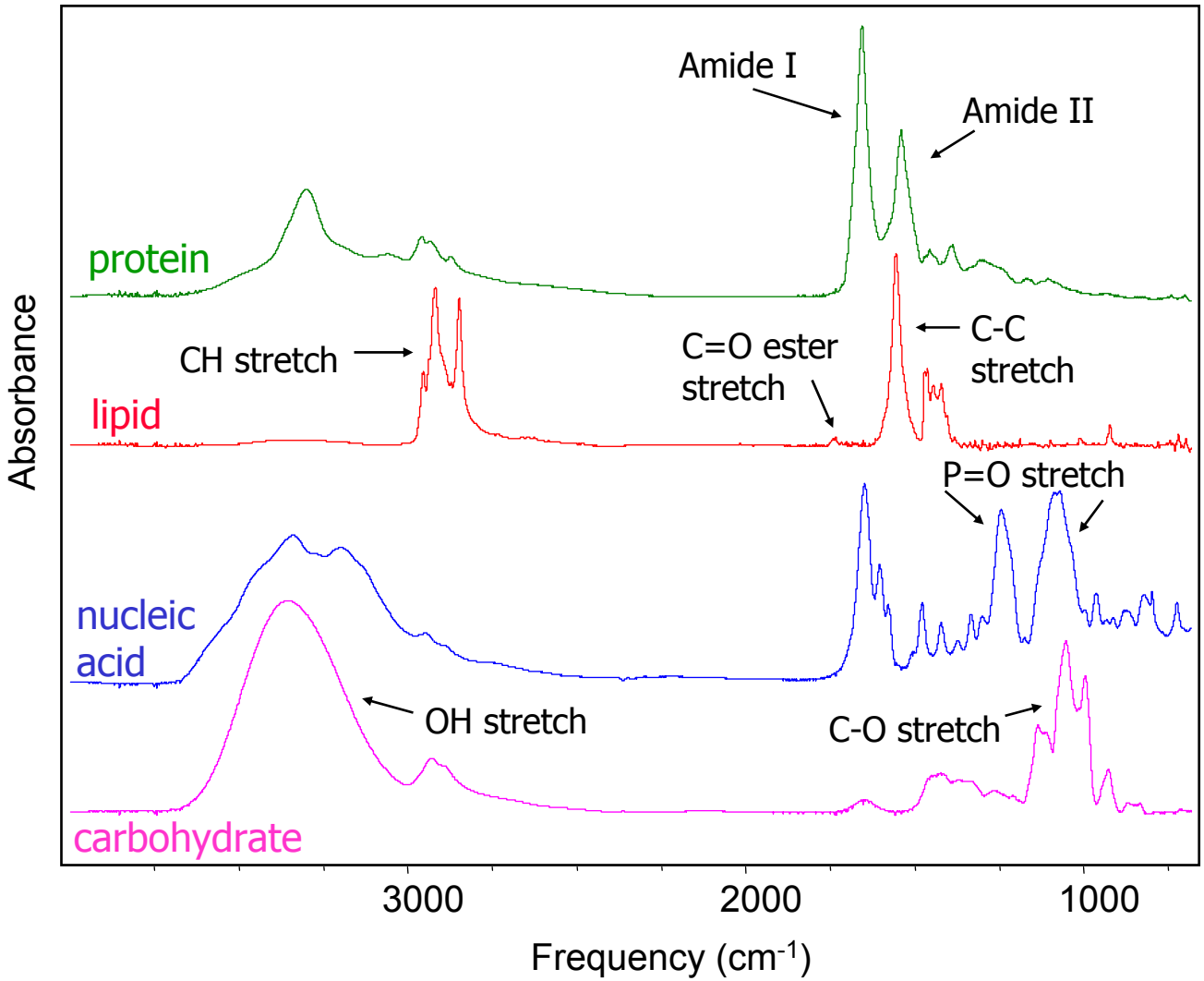


Nucleic Acid

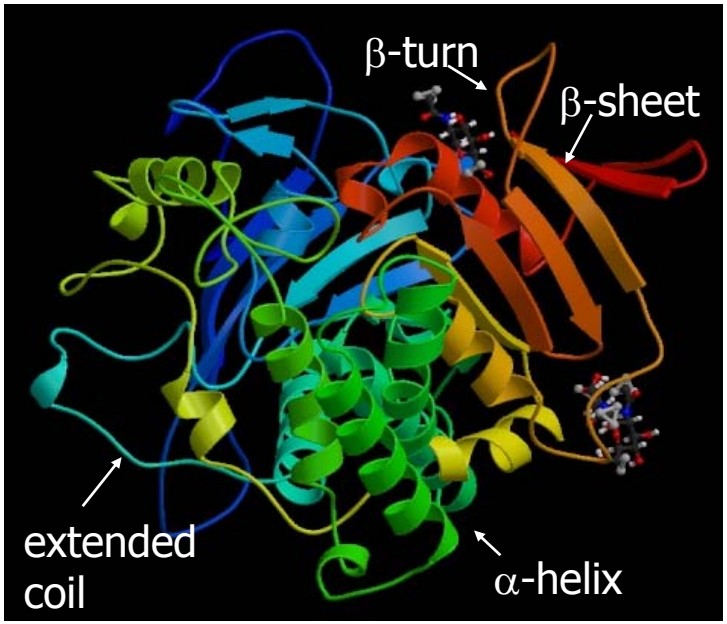


Carbohydrate

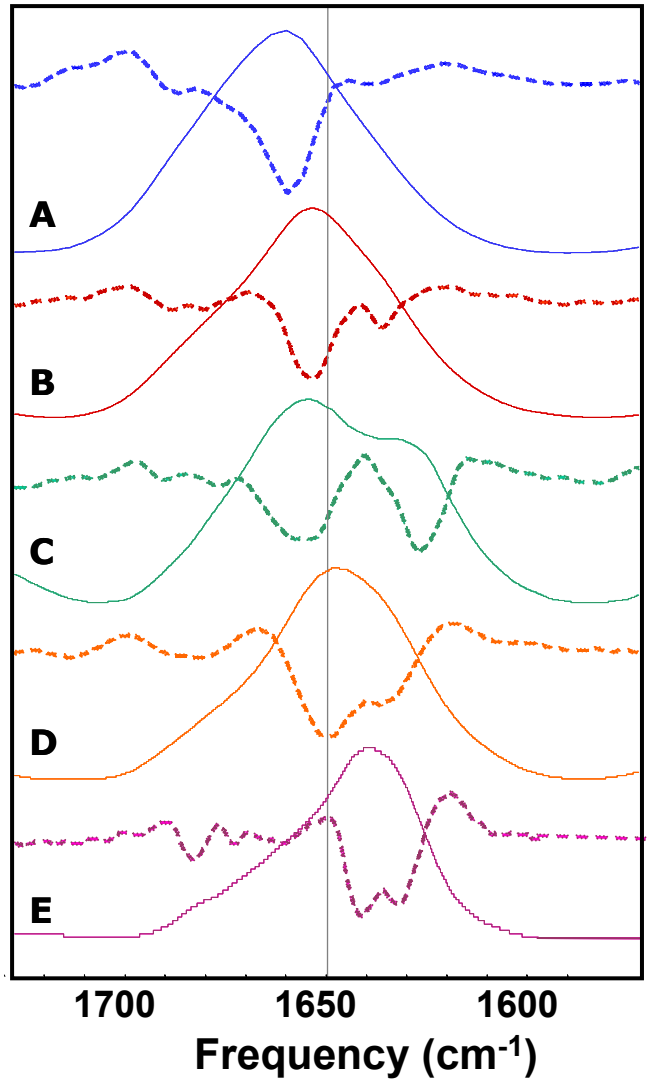




Protein Structure Determination with FTIR



Amide I Secondary Structure Assignments:	
1620 - 1640	β-sheet
1645	extended coil (D_2O)
1648 - 1657	α-helix
1660	triple helix
1670 - 1695	β-sheet, β-turn



Collagen (triple helix)

Myoglobin (α-helix)

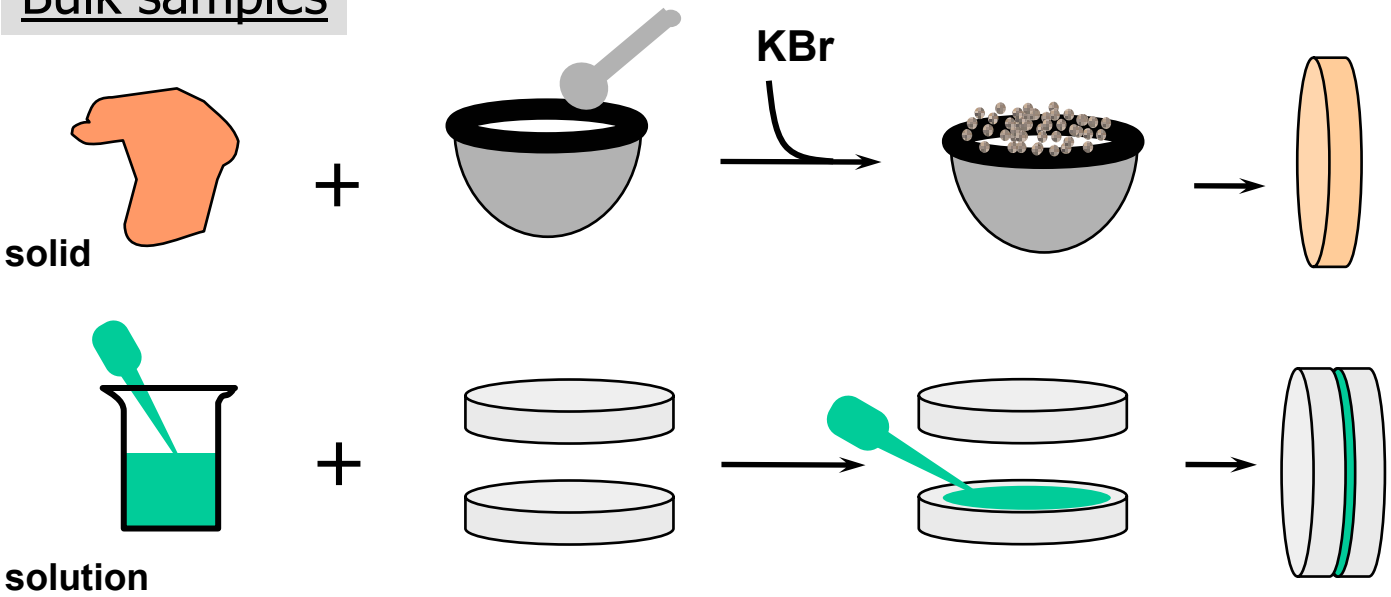
Abeta Amyloid (α-helix + β-sheet)

Cytochrome c (α-helix + coil)

STI (β-sheet + coil)

IR Spectroscopy vs. Micro-Spectroscopy

Bulk samples



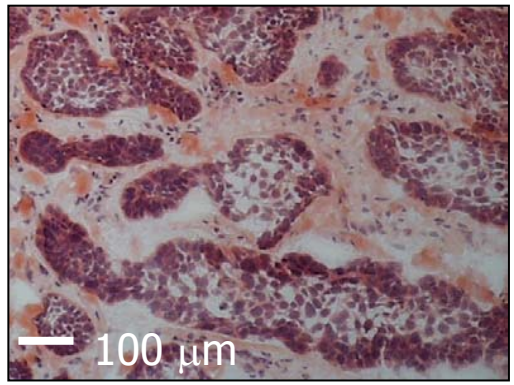
Spatial Resolution:
~1-3 mm

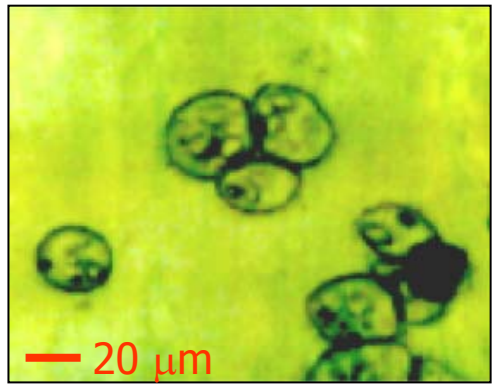
Microscopic Heterogeneity

Spatial Resolution:
~ 25-30 μm (globar)
~ 5-10 μm (synchrotron)

Why SYNCHROTRON Infrared Microspectroscopy?

Biological sample are SMALL:
- spatially
- in concentration





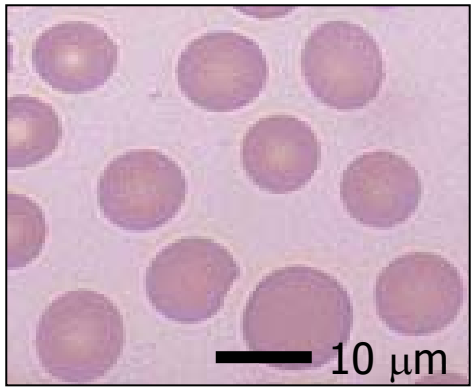
Synchrotron Advantages:

BRIGHTNESS (1000x)
- Higher Spatial Resolution
- Better Signal-to-Noise
- Faster Data Collection

+

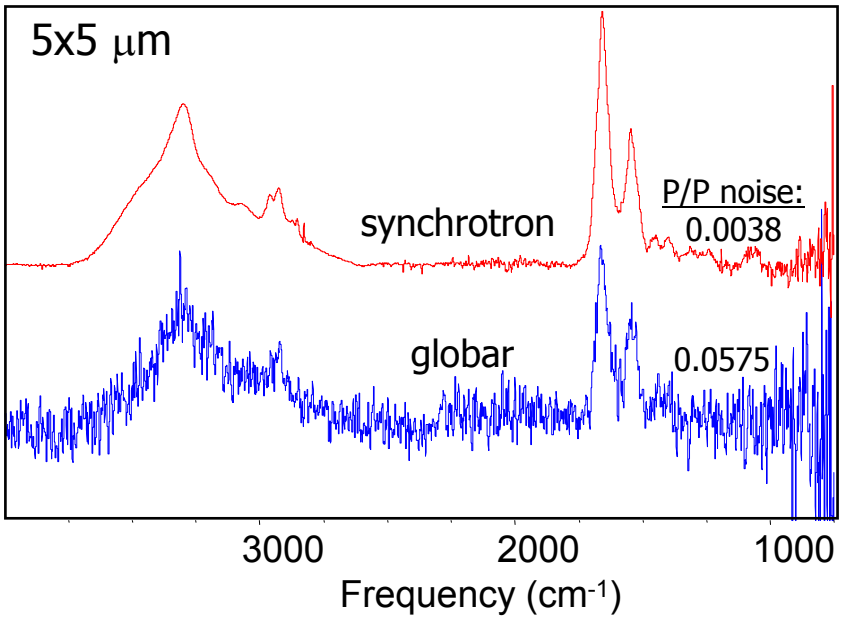
BROADBAND
- Spectroscopy

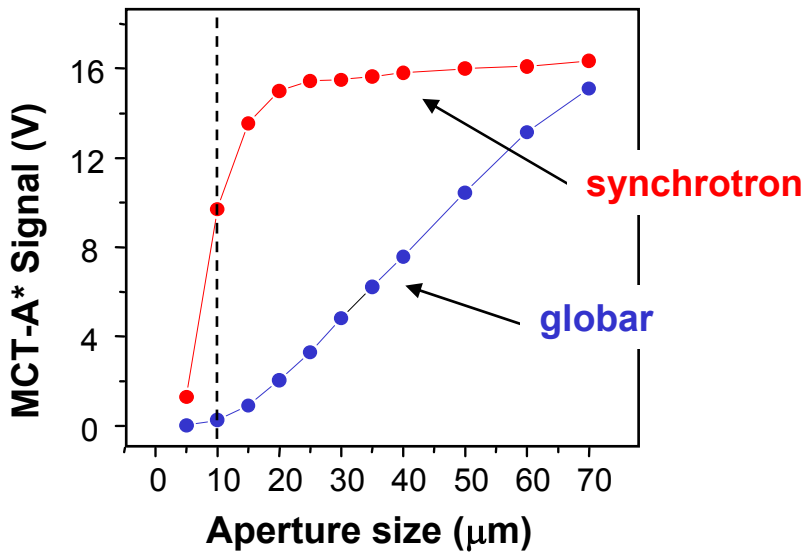
Single Red Blood Cell: Globar vs. Synchrotron Source



Diffraction Limit	
4000 cm^{-1}	2.5 μm
2950 cm^{-1}	3.4 μm
1650 cm^{-1}	6.1 μm
1200 cm^{-1}	8.3 μm
1000 cm^{-1}	10 μm
600 cm^{-1}	17 μm
200 cm^{-1}	50 μm

- Synchrotron IRMS is diffraction-limited
- Conventional IRMS is throughput-limited





Beamline U10B

Synchrotron infrared beam pipe

Nicolet Magna 860 FTIR

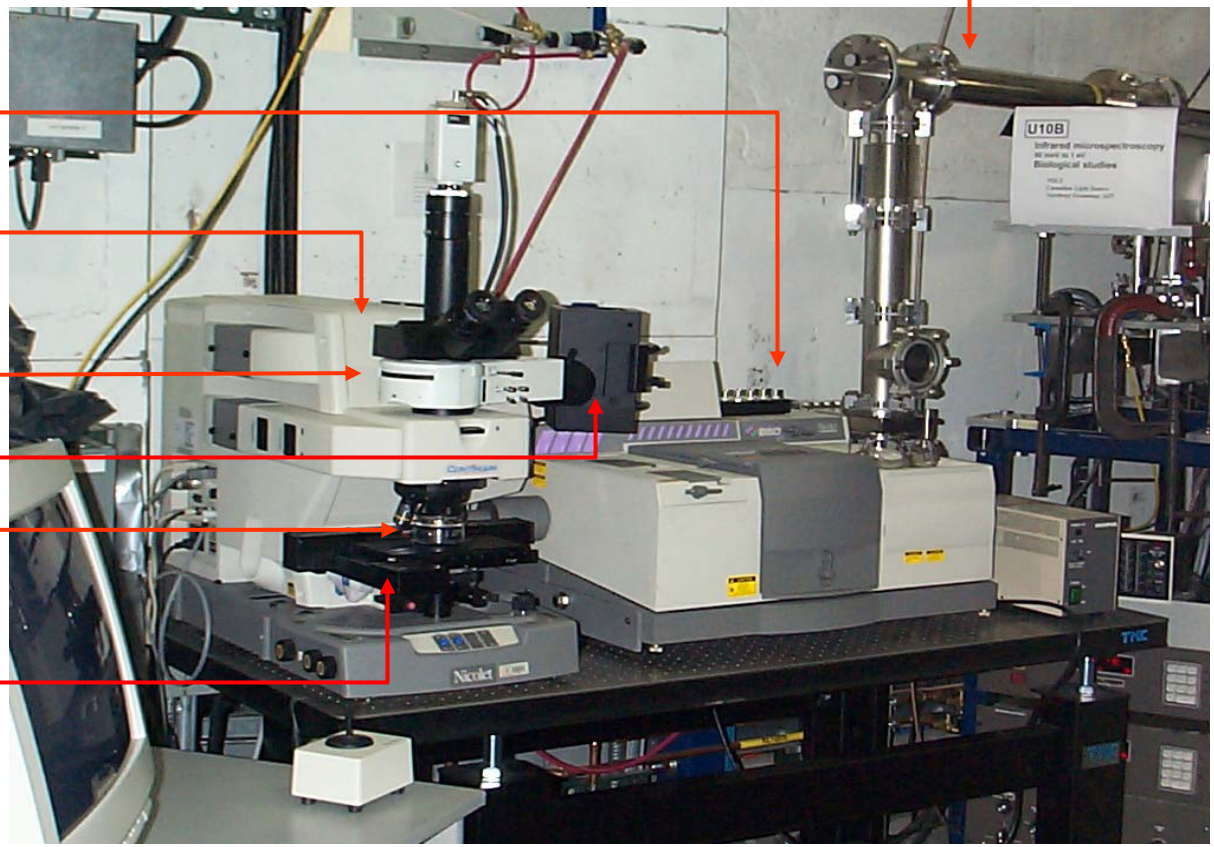
Spectra Tech Continuμm IR microscope

Filter cube turret

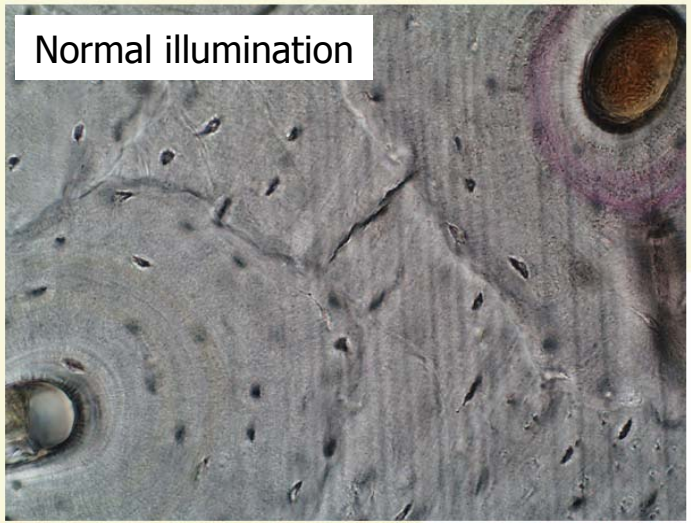
UV (quartz) light source

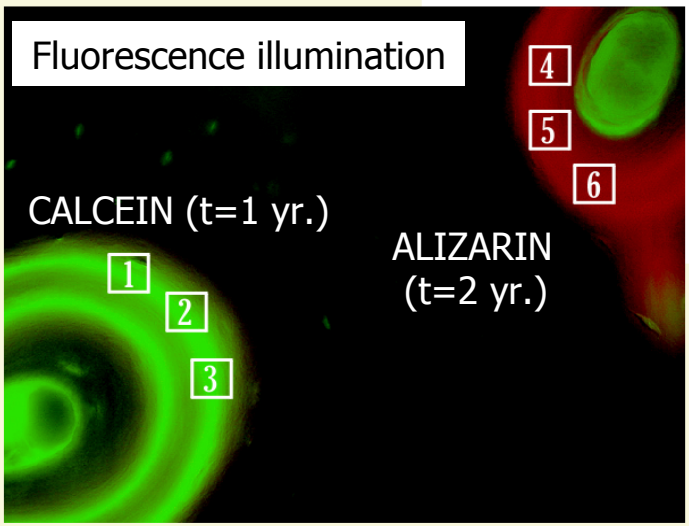
Schwarzchild 32x IR objective

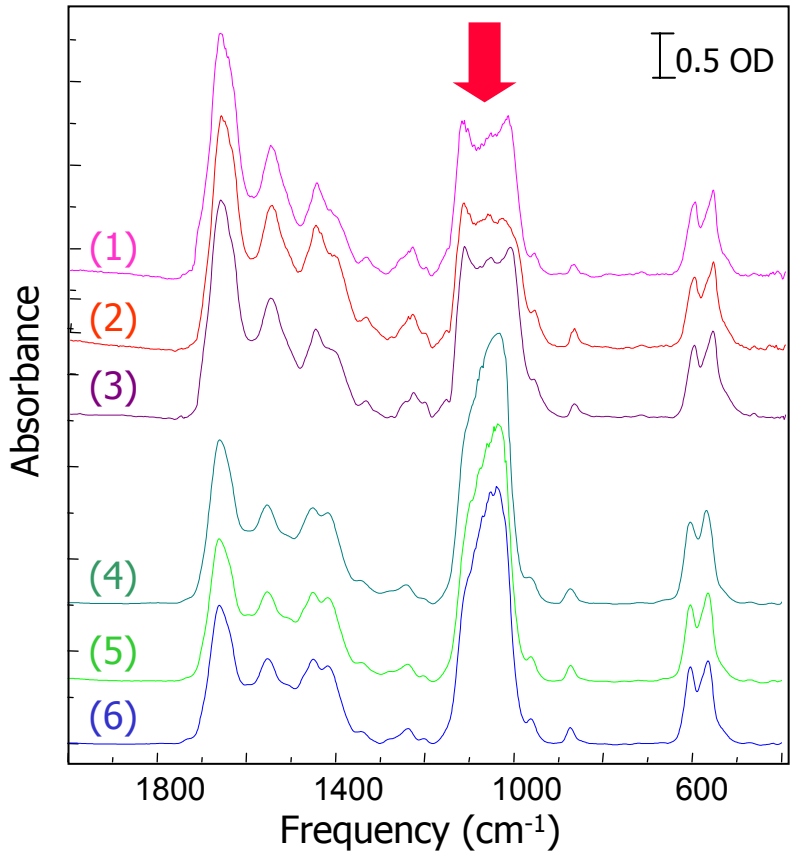
Automated mapping stage



Chemical Content of New Bone in Osteoporosis



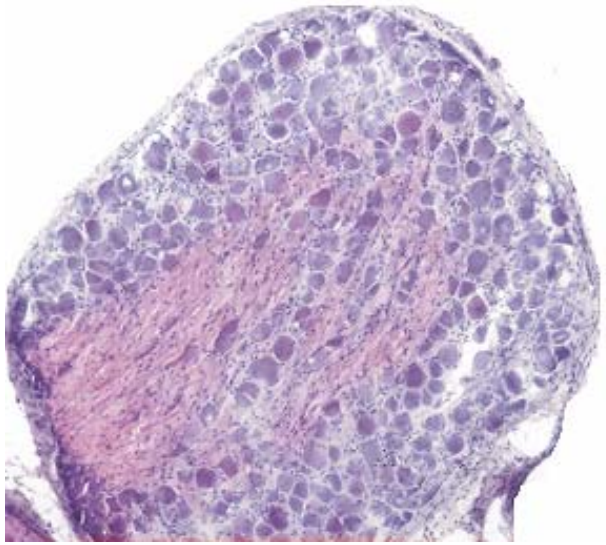




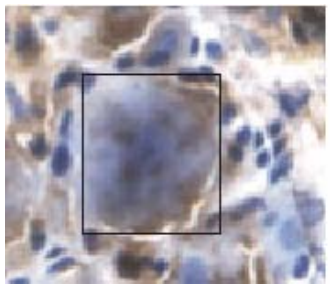
- **Rate of bone mineral formation is slower in osteoporosis**

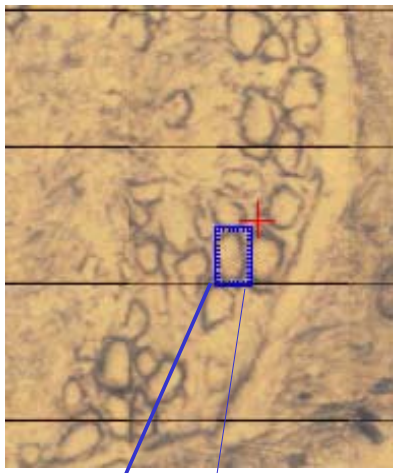
L.M. Miller, J. Tibrewala, C.S. Carlson. *Cell. Mol. Biol.*, **46**:1035-44 (2000).

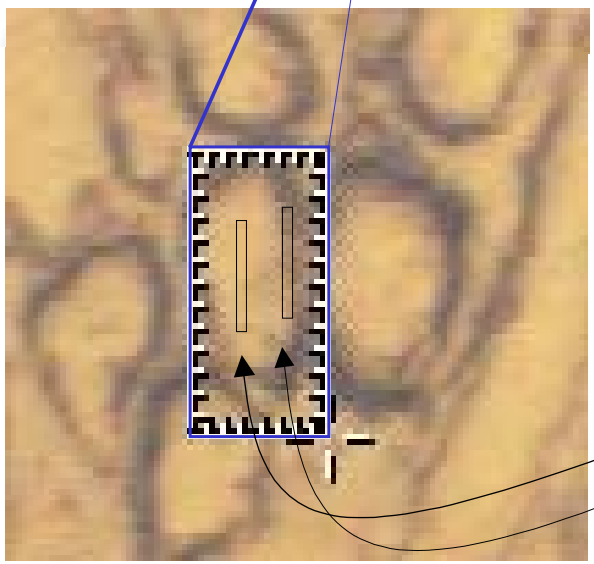
Prion Protein Structure and Location

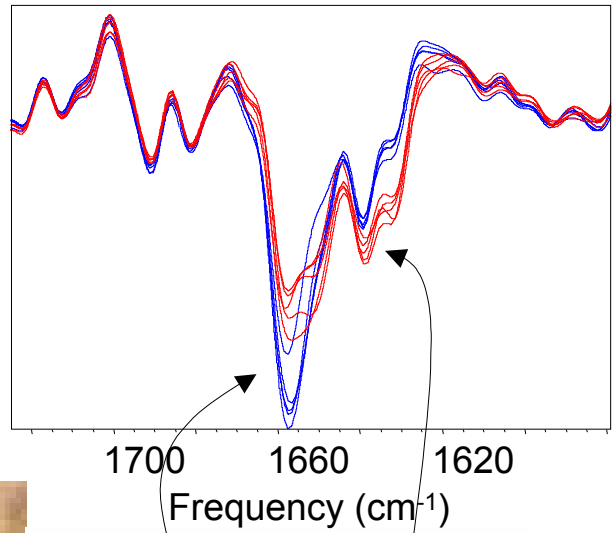


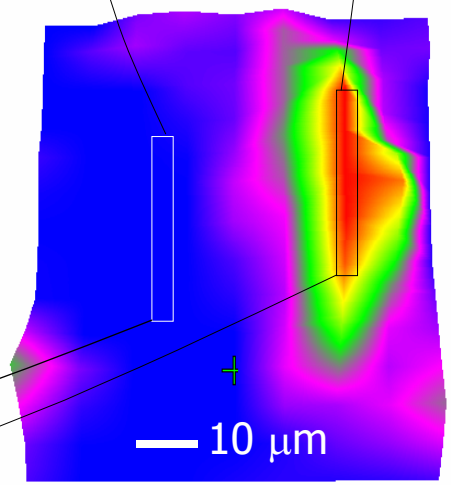
- Hamster scrapie 263K
- Dorsal root ganglia
- Terminally infected



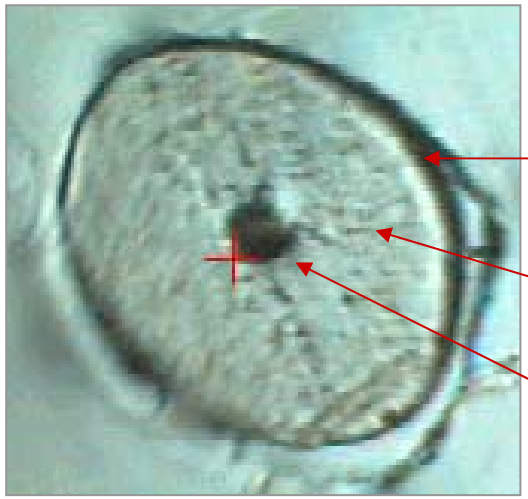








Chemical Imaging of Hair

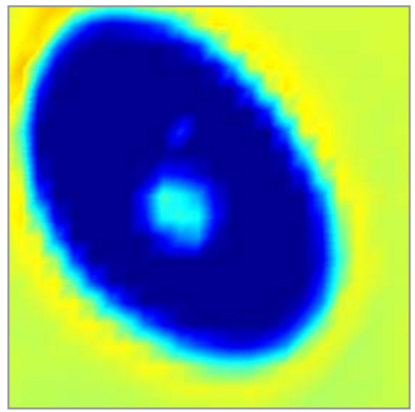


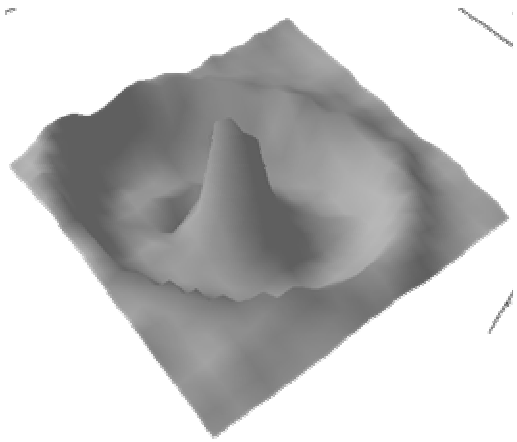
cuticle (3-5 μm)

cortex (40-100 μm)

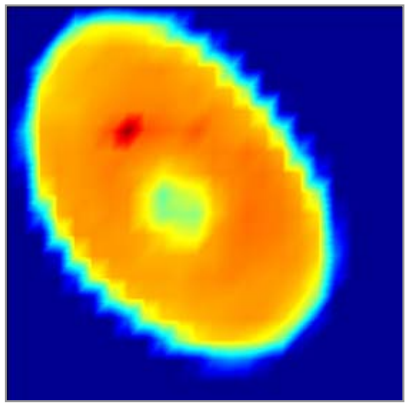
medulla (5-10 μm)

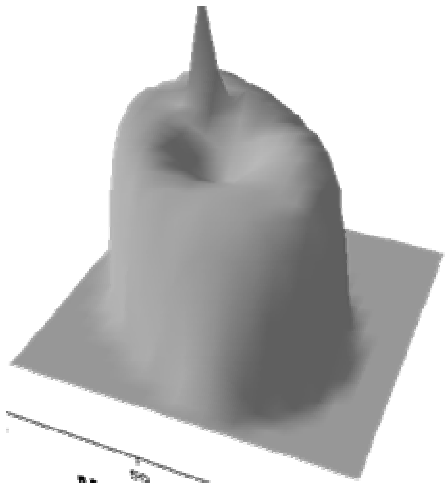
Phospholipids



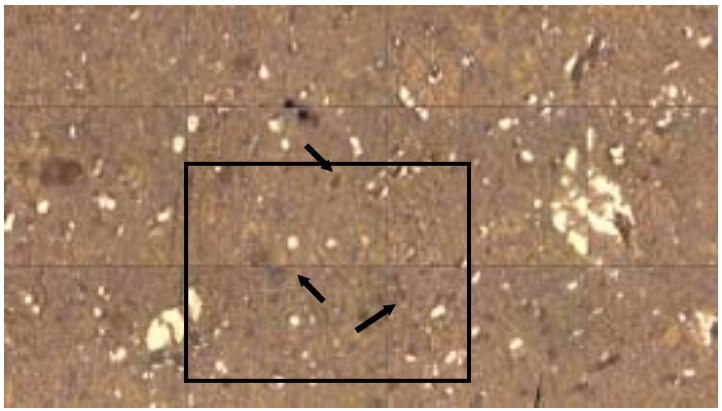


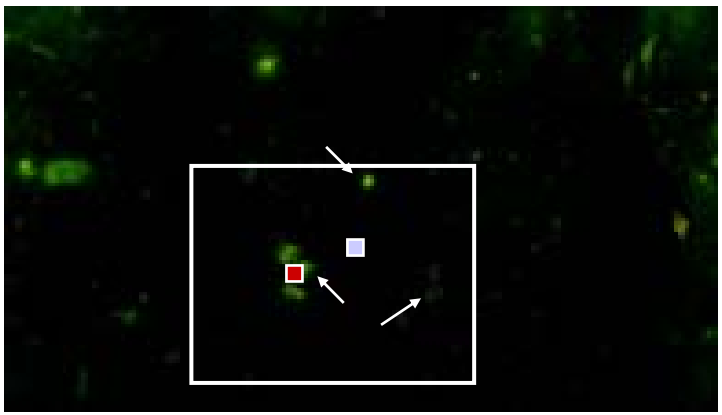
Proteins

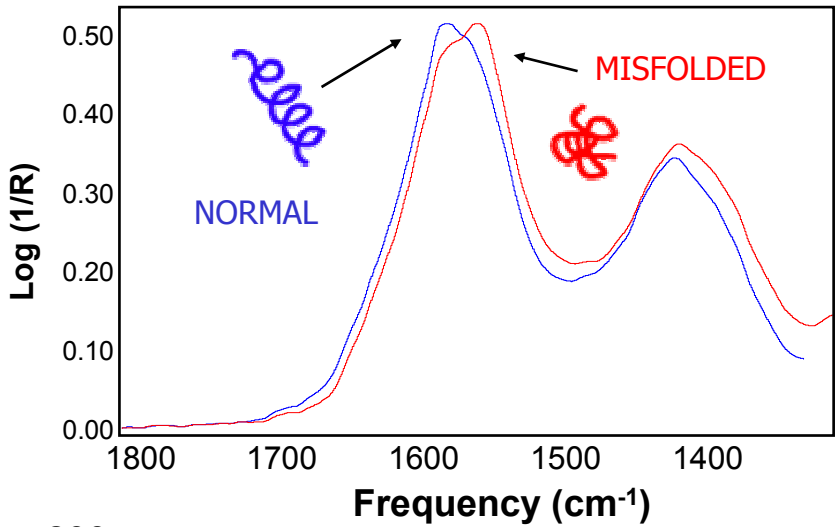


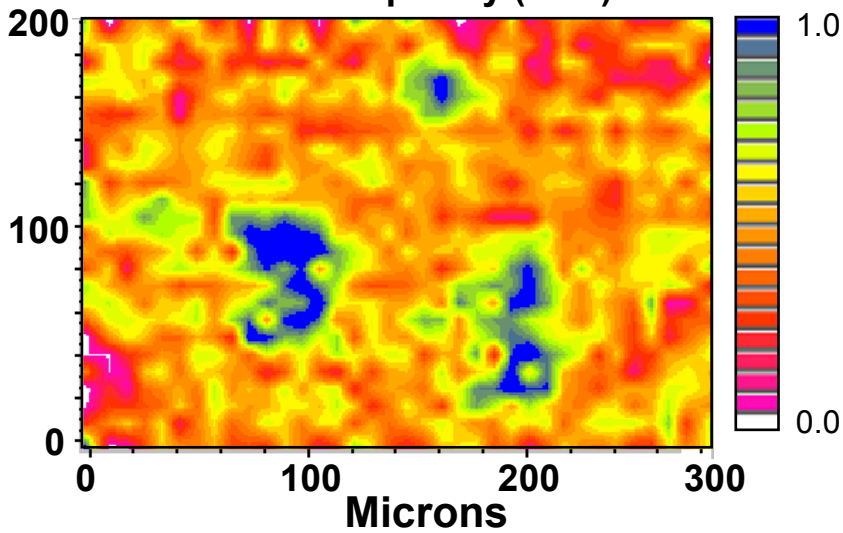


Protein Aggregate Formation in Alzheimer's Plaques





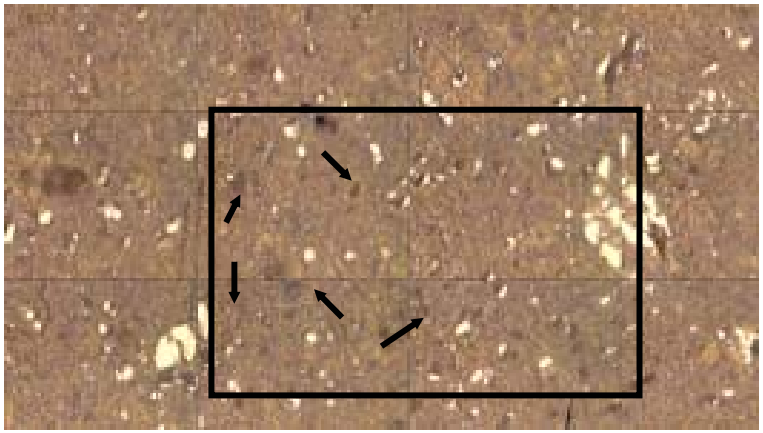


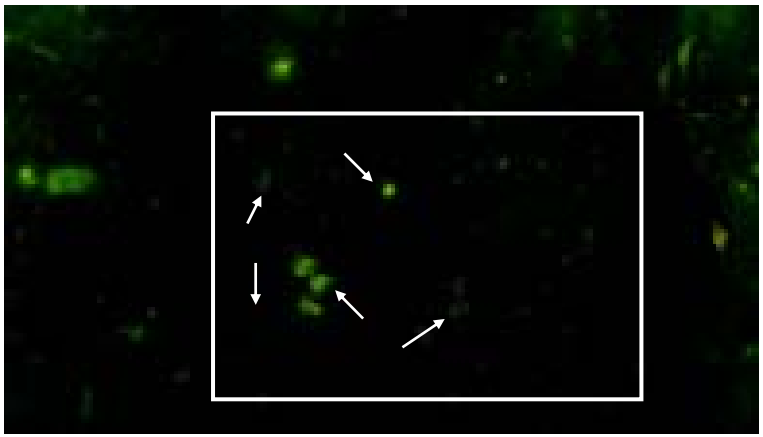


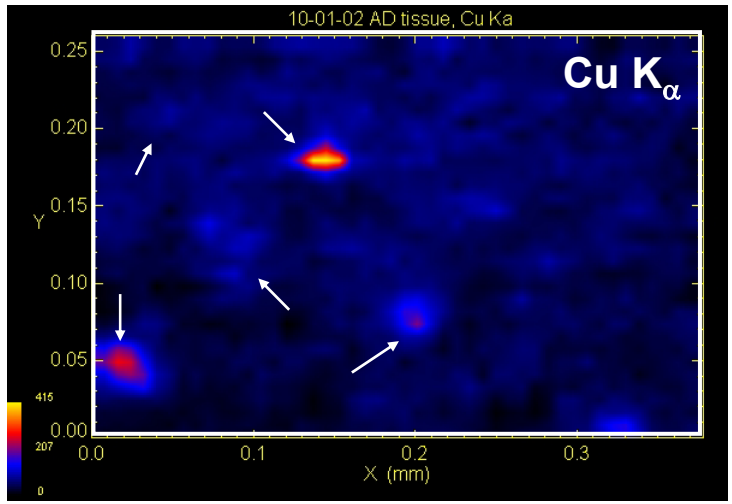
- misfolded, aggregated protein is associated with Abeta plaques

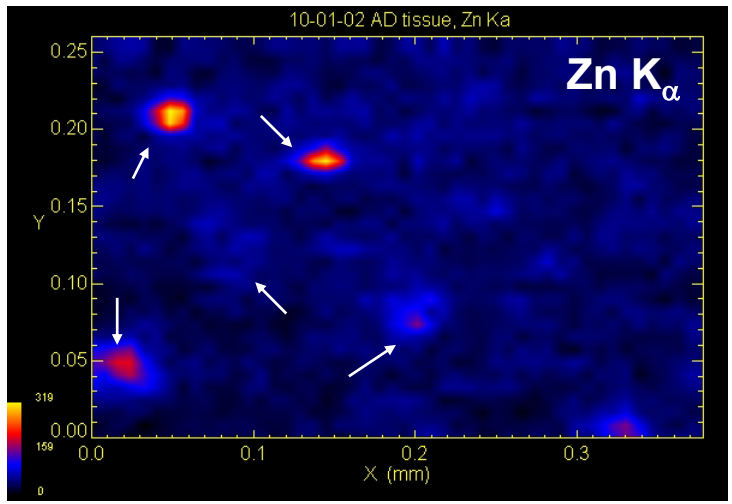
L.M. Miller, P. Dumas, N. Jamin, J.-L. Teillaud, J. Miklossy, L. Forro (2002). *Rev. Sci. Instr.*, **73**: 1357-60.

Metal Accumulation in Alzheimer's Plaques



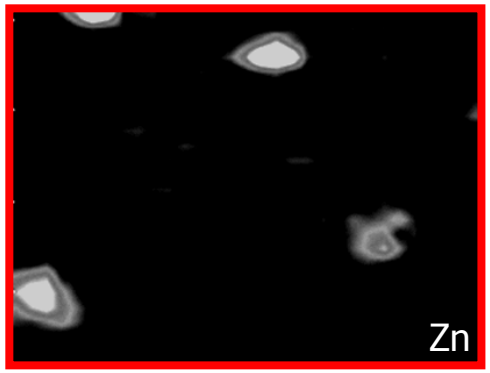


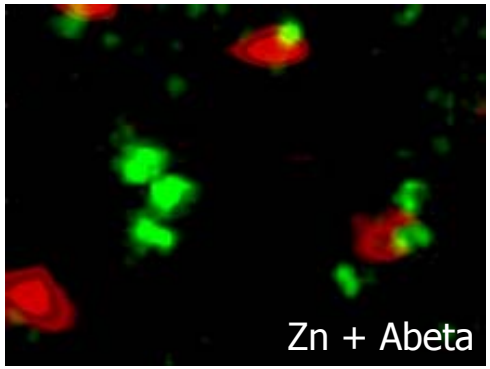


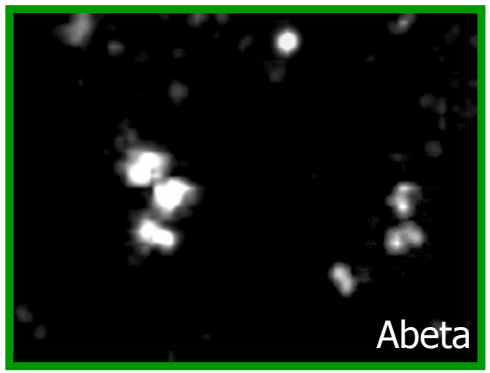


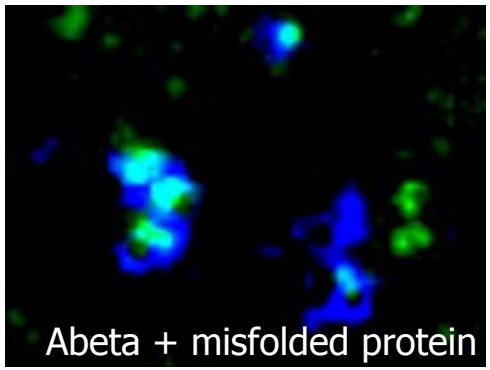
- Zn and Cu are associated with Abeta plaques

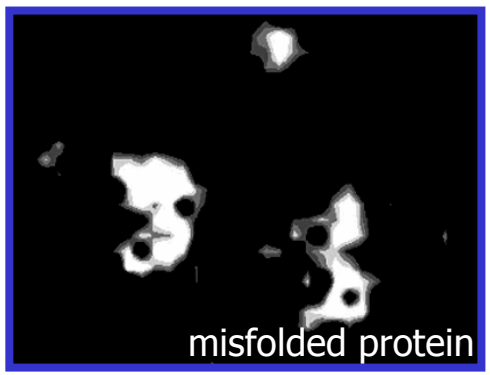
Correlation Between Metal Accumulation and Protein Aggregation

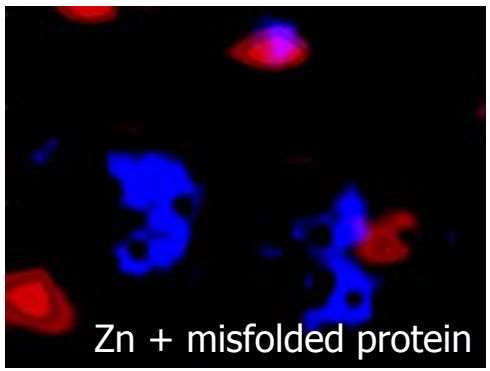


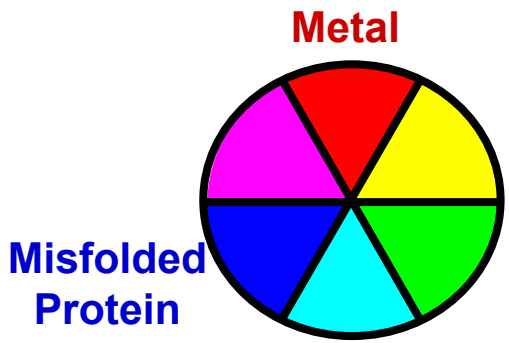


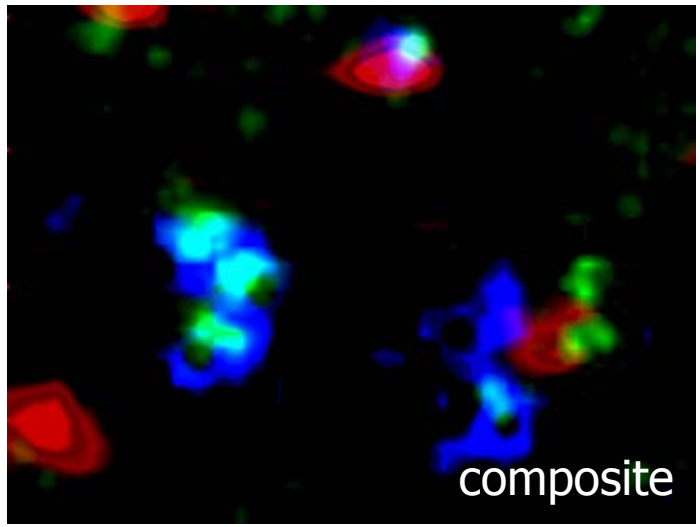




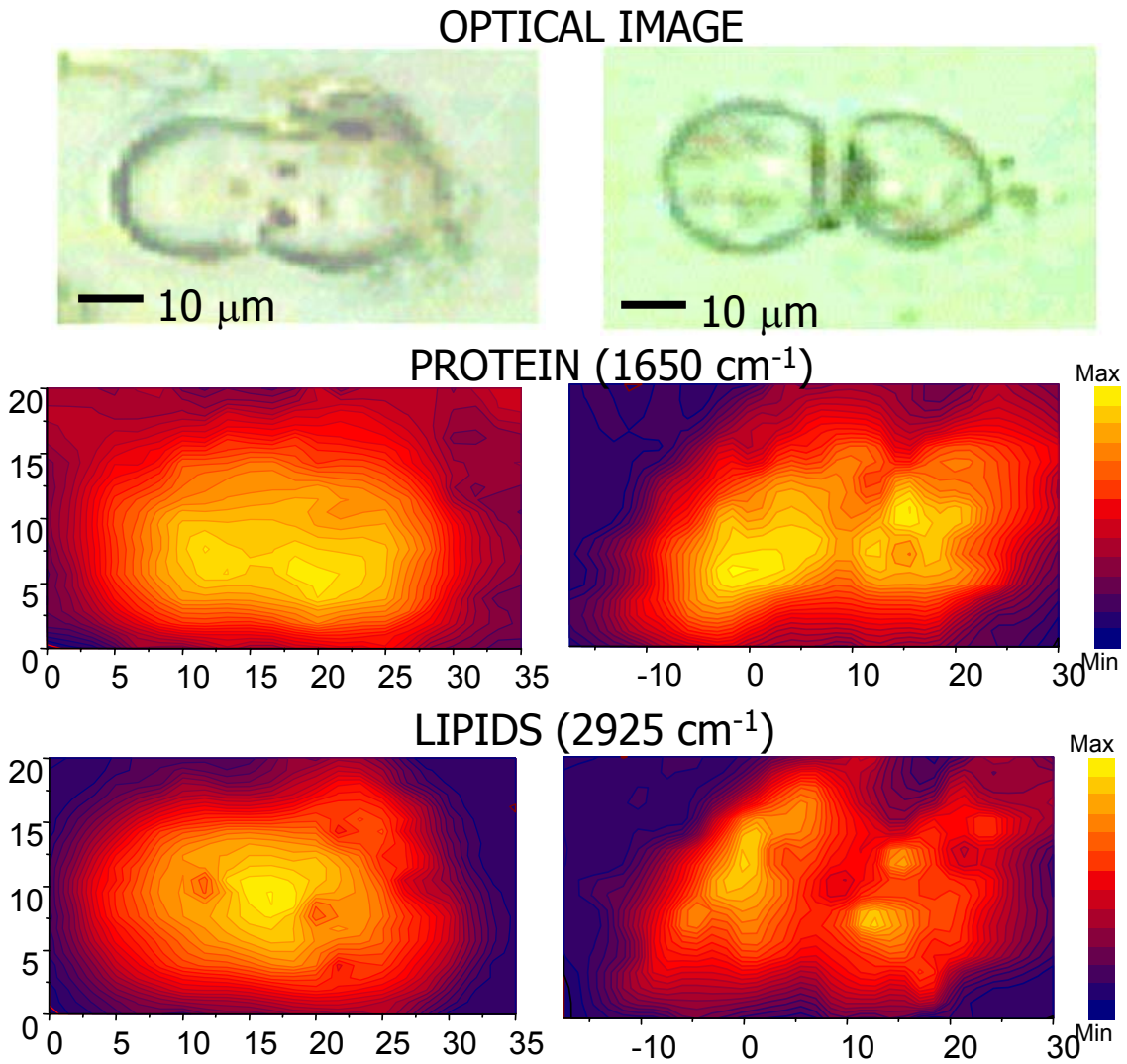






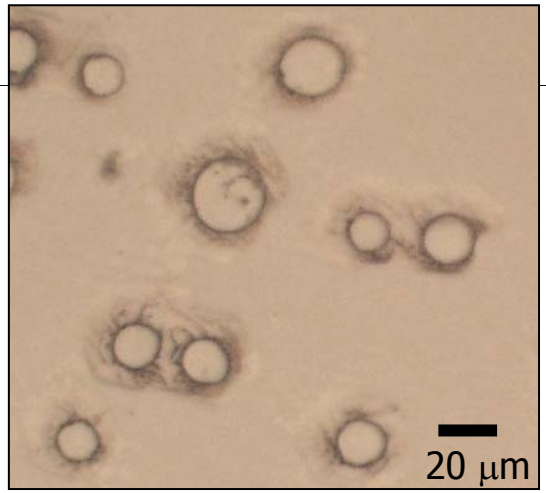


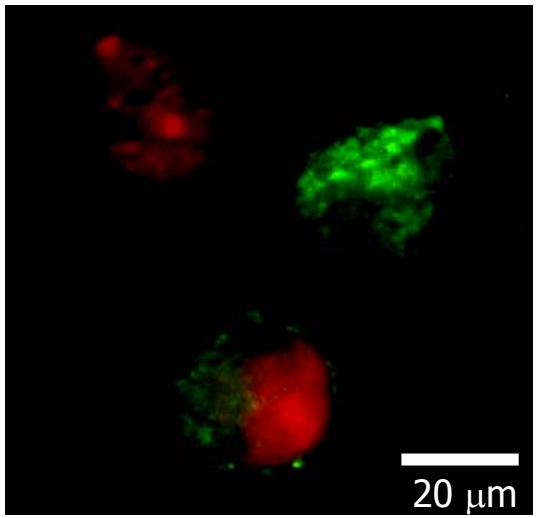
Chemical Imaging of Mitotic Cells

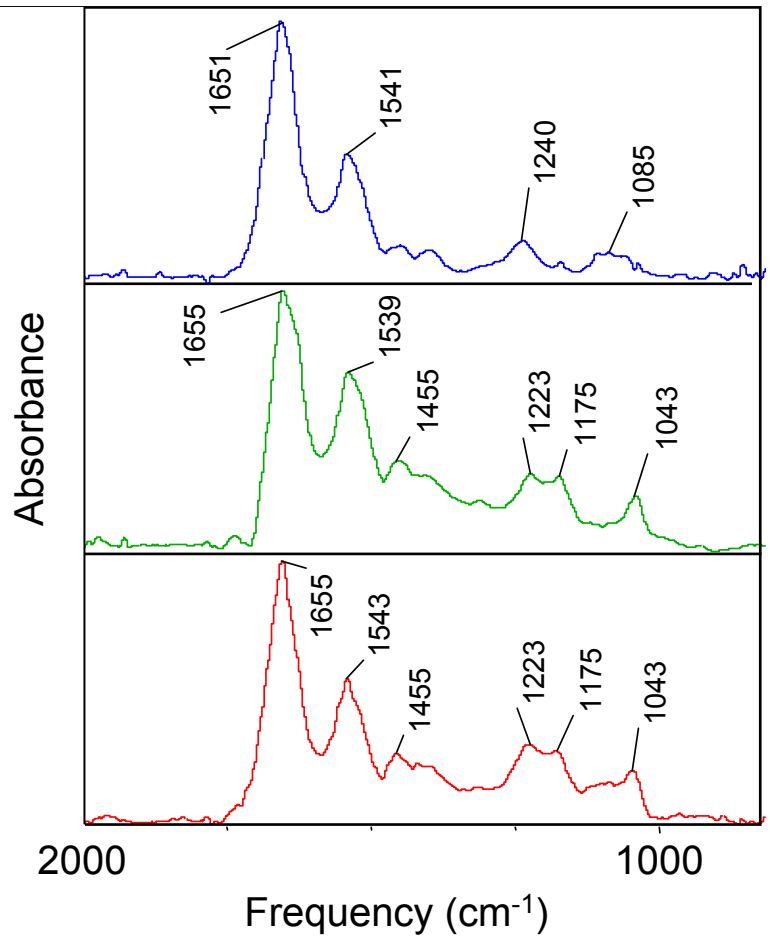


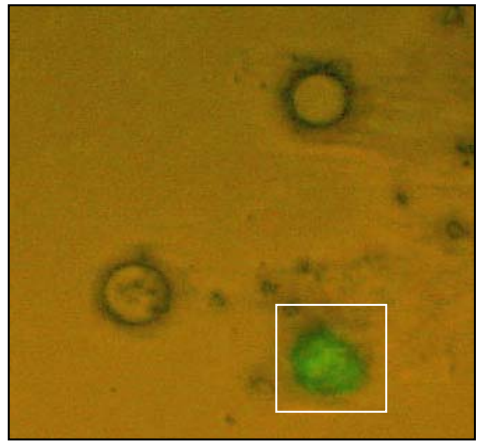
N. Jamin, J.L. Teillaud, P. Dumas, G.L. Carr, G.P. Williams (1998). *PNAS* **95**: 4837-40.

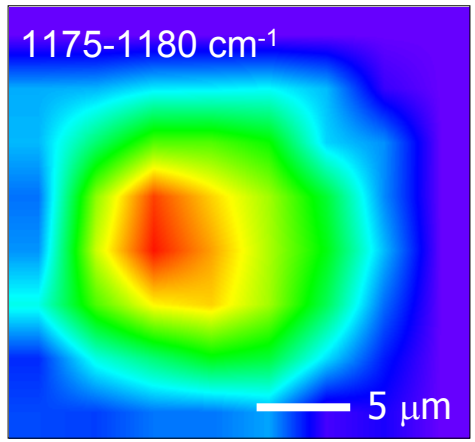
Chemical Changes in Apoptotic Cells









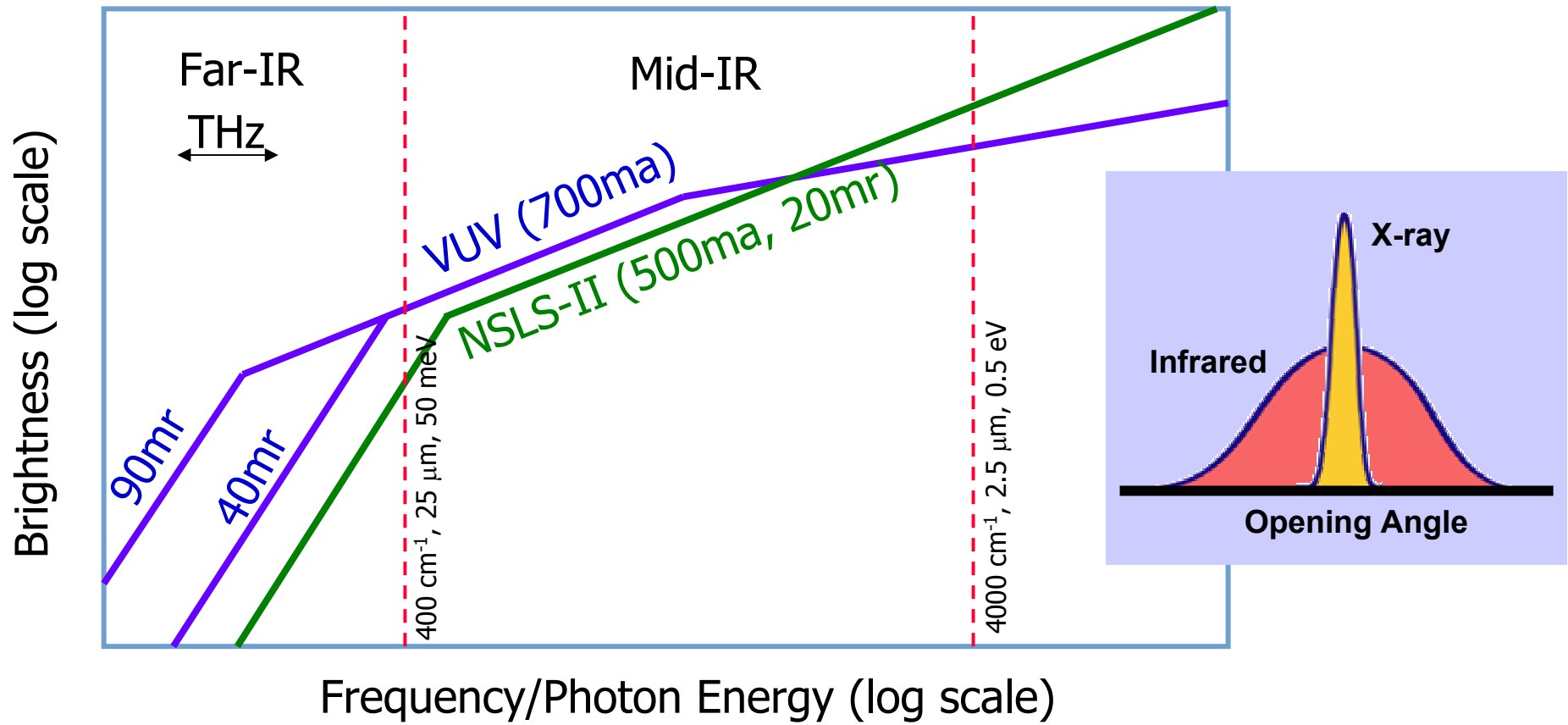


How does apoptosis proceed in real time?

L.M. Miller, P. Dumas, N. Jamin, J.-L. Teillaud, J. Miklossy, L. Forro (2002). *Rev. Sci. Instr.*, **73**: 1357-60.

Impact of NSLS-II: Schematic Brightness Comparison

IR flux is primarily dependent upon ring current in mid-IR



- **Mid IR: NSLS-II will have equal brightness to NSLS**
- **Far-IR and multi-point imaging: disadvantaged by NSLS-II**

Summary

Overview

- Infrared microspectroscopy and imaging can be used to image the chemical makeup of biological tissues and cells
- The spatial resolution of synchrotron IRMS is diffraction-limited at 2-20 μm

Importance of Research

- Chemical imaging of mineralized tissues, *in situ* protein, lipid, nucleic acid content, protein structure

Scientific Challenges and Opportunities

- Multi-technique imaging: combining x-ray, visible, IR imaging techniques
- Improve spatial resolution
- Improve data collection rates

Impact of NSLS-II

- NSLS-II brightness will slightly improve the mid-IR imaging capabilities
- NSLS-II will prevent the creation of large opening-angle beamlines for (1) far-IR applications, and (2) line-source (i.e. multi-point) imaging

Synergy and Demand

- Combination of IRMS with x-ray imaging techniques
- Currently 4 IRMS bending magnet beamlines; need at least this many for NSLS-II